Restoration of Microbial Residues in Soils of the Conservation Reserve Program

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ABSTRACT

To elucidate the role of microorganisms for C and N sequestration in arable soils converted to grassland (sites of the Conservation Reserve Program; CRP), we determined amino sugars as indicators for microbial residues in surface samples (0–5 cm) obtained from each of 10 adjacent native grassland, CRP, and cropland sites across the U.S. Great Plains. The CRP sites were 6 to 10 yr and the cropland sites were >80 yr old. Compared with native grasslands, the CRP sites had lost between 17 and 50% and the cropland sites between 32 and 94% of their surface soil organic matter (SOM). The C/N ratio was not significantly different among the three land-use systems, indicating that C and N losses occurred at similar intensity. The mean amino sugar concentrations decreased in the order native grassland (70 g kg^{-1} C; 750 g kg^{-1} N) > CRP (53 g kg^{-1} C; 570 g kg^{-1} N) > cropland (47 g kg^{-1} C; 450 g kg^{-1} N). This decrease in the element-normalized concentrations of amino sugars indicated that they responded faster to management than other C or N containing compounds. The response of individual amino sugars related to soil compaction and the temperature regime. We suggest that the resequestration of C and N into the residues of bacteria and fungi requires several years, but as it depends on land use it could be manipulated using, for example, soil decompacting techniques to improve CRP efficiency.

Past conventional tillage practices are estimated to have accounted for about two-thirds of total soil CO_2 emissions (Lal et al., 1998). The CRP, authorized by the U.S. Food Security Act of 1985, was established to protect eligible land from further degradation (Council for Agricultural Science and Technology, 1992). This program is designed to encourage farmers to plant long-term resource-conserving covers, such as grasses, in formerly eroded arable soil of the prairie (USDA, 2001). Following its implementation, a major additional benefit of the program became recognized, namely that land placed in the CRP has potential to offset U.S. emissions of CO_2 by sequestering carbon in soil (Bruce et al., 1999; Lal et al., 1999; Follett et al., 2001). It has been estimated that the 13.8 million ha of CRP land may sequester between 10 and 25 Tg of C as soil organic C across a 10-yr period (Lal et al., 1998). Less is known about N sequestration as soil organic N, and about the mechanisms regulating the restoration of SOM levels at CRP compared with degraded arable sites.

As plants do not synthesize amino sugars in significant amount (Parsons, 1981; Stevenson, 1982), and because amino sugars are rather stable against fluctuations in enzyme activities and living microbial biomass (Nannipieri et al., 1979; Chantigny et al., 1997; Guggenberger et al., 1999), the concentration of amino sugars is a useful indicator for microbially sequestered C and N. Bacterial sequestration of C and N results in an accumulation of muramic acid and glucosamine-containing cell wall residues, while accumulating galactosamine frequently originates from actinomycetes and capsular and extracellular saccharides of the bacteria. In contrast, fungal sequestration of C and N results in an amino sugar production that almost exclusively comprises glucosamine. The chitin of arthropod exoskeleton does not significantly contribute to the amino sugar content in soil, due to the low biomass of soil arthropods relative to that of fungi and bacteria (for reviews, see Sharon, 1965; Parsons, 1981; Amelung, 2001).

The contribution of amino sugars to the soil C or N reserves of the prairie depended on the soil temperature regime (Amelung et al., 1999). In addition, long-term cropping resulted in increased amino sugar losses, primarily of those originating from bacteria (Zhang et al., 1997). How fast this microbe-derived C and N pool reacted to land-use changes, and whether its depletion may be easily reversed upon CRP practice remained unknown. The objective of this study was to investigate the impact of CRP practice on the restoration of the soil amino sugar pool at the North American prairie in different climatic regions.

MATERIALS AND METHODS

Samples

Ten sites from different climatic regions were selected for this investigation across the historical grasslands of the U.S. prairies (Table 1). Soil surface texture ranged from sand to silty-clay loam, and soil pH from 4.1 to 7.5 in 0.01 *M* $CaCl_2$. At each site, composite samples were collected from (i) a native prairie, (ii) an adjacent long-term cropland (>80 yr cultivated), and (iii) one adjacent CRP site (cropland that was put back to native grass vegetation 6–10 yr ago). This paired-plot design allowed site factors, other than land use, to be kept as constant as possible. Soils were classified using soil taxonomy (Soil Survey Staff, 1998). Composite samples were collected from the 0- to 5-cm depth from each side of excavated soil pits. Samples were also taken from the 5- to 10-cm depth intervals and then by genetic horizon to a depth of ≈2 m (data not shown). The sampling times were: June 1996 for all sites in Minnesota; July 1997 for all sites in North Dakota; September 1994 for all sites in Iowa and Nebraska; September 1995 for the sites in Missouri; April 1995 for the sites in Texas; and April 1997 for all sites in Oklahoma. All samples were stored air-dried and ground to less than 2 mm prior to analysis. For more information on site properties, see Follett et al. (2001).

Analysis

Total C and N were determined after dry combustion by measurement of evolved CO_2 (method 6A2) and N_2 (method

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Published in Soil Sci. Soc. Am. J. 65:1704–1709 (2001).

Abbreviations: CRP, Conservation Reserve Program; MAT, mean annual temperature; SOM, soil organic matter.

Table 1. Studied sites (0- to 5-cm sampling depth).

Locality	Series	Soil type†	MAT‡	MAP§	Land use	pH_{CaCl_2}	Clay; Silt	Bulk density	total C	total N	Amino sugars
			°C	cm			g kg^{-1}	g cm^{-3}	— g kg^{-1} soil —		mg kg^{-1}
Minnesota (S)	Nicollet	Fine-loamy, mixed, superactive, mesic Aquic Hapludoll	3	62	Native	6.1	263; 439	0.89	63.9	4.96	4060
	Nicollet	Fine-loamy, mixed, superactive, mesic Aquic Hapludoll			CRP (9 yr)¶	5.8	200; 300	1.33	33.8	2.93	1960
	Nicollet	Fine-loamy, mixed, superactive, mesic Aquic Hapludoll			Crop	5.6	217; 370	1.32	29.8	2.96	1280
North Dakota (W)	Farnuf	Fine-loamy, mixed, superactive, frigid Typic Argiustoll	5	39	Native	6.0	200; 470	0.66	50.7	4.39	2320
	Farnuf	Fine-loamy, mixed, superactive, frigid Typic Argiustoll			CRP (10 yr)	6.4	226; 282	1.29	26.9	3.05	380
	Farnuf	Fine-loamy, mixed, superactive frigid Typic Argiustoll			Crop	5.5	208; 343	0.95	28.5	2.88	360
North Dakota (E)	Barnes	Fine-loamy, mixed, superactive, frigid Calcic Haplustoll	5	49	Native	6.5	290; 465	0.70	95.5	8.38	6480
	Barnes	Fine-loamy, mixed, superactive, frigid Calcic Haplustoll			CRP (10 yr)	7.3	245; 342	1.02	37.3	3.80	2510
	Barnes	Fine-loamy, mixed, superactive, frigid Calcic Haplustoll			Crop	7.1	276; 320	1.00	35.6	3.55	2200
Minnesota (2nd N)	Percy	Coarse-loamy, mixed, superactive, frigid Typic Calciaquoll	7	72	Native	7.2	252; 289	1.24	49.7	4.06	3130
	Percy	Coarse-loamy, mixed, superactive, frigid Typic Calciaquoll			CRP (7 yr)	7.4	153; 220	1.58	50.9	3.78	2500
	Percy	Coarse-loamy, mixed, superactive, frigid Typic Calciaquoll			Crop	7.5	150; 216	1.03	25.1	1.98	730
Iowa	Macksburg	Fine, smectitic, mesic Aquic Argiudoll	10	84	Native	6.1	300; 660	0.81	56.5	4.75	3700
	Sharpsburg	Fine, smectitic, mesic Typic Argiudoll			CRP (8 yr)	6.9	310; 650	1.09	35.6	2.86	1870
	Sharpsburg	Fine, smectitic, mesic Typic Argiudoll			Crop	5.7	300; 670	1.24	28.4	2.71	1190
Nebraska	Crete	Fine, smectitic, mesic Pachic Argiustoll	11	72	Native	5.5	220; 590	0.58	99.1	9.51	7950
	Hastings	Fine, smectitic, mesic Udic Argiustoll			CRP (6 yr)	5.0	330; 580	1.19	23.9	2.18	1540
	Hastings	Fine, smectitic, mesic Udic Argiustoll			Crop	5.0	390; 530	1.35	15.1	1.60	980
Missouri	Mexico	Fine, smectitic, mesic Aeric Vertic Epiaqualf	12	99	Native	4.4	213; 752	0.84	43.2	3.87	3540
	Mexico	Fine, smectitic, mesic Aeric Vertic Epiaqualf			CRP (7 yr)	6.8	211; 701	1.29	19.5	1.89	650
	Mexico	Fine, smectitic, mesic Aeric Vertic Epiaqualf			Crop	5.0	243; 687	1.35	1.74	1.86	990
Texas	Pullman	Fine, mixed, superactive, thermic Torrertic Paleustoll	14	50	Native	6.3	280; 525	1.01	39.3	4.03	2460
	Pullman	Fine, mixed, superactive, thermic Torrertic Paleustoll			CRP (8 yr)	6.1	284; 486	1.36	12.5	1.43	840
	Pullman	Fine, mixed, superactive, thermic Torrertic Paleustoll			Crop	6.1	306; 502	1.28	7.3	0.95	410
Oklahoma (E)	Stephenville	Fine-loamy, siliceous, active, thermic Ultic Haplustalf	16	103	Native	5.6	95; 277	1.26	13.9	1.40	1000
	Stephenville	Fine-loamy, siliceous, active, thermic Ultic Haplustalf			CRP (10 yr)	5.0	63; 175	1.50	7.3	0.79	470
	Stephenville	Fine-loamy, siliceous, active, thermic Ultic Haplustalf			Crop	4.1	35; 100	1.27	4.5	0.53	250
Oklahoma (W)	Madge	Fine-loamy, mixed, superactive, thermic Typic Argiustoll	16	85	Native	7.4	158; 392	1.33	10.7	1.26	1000
	Madge	Fine-loamy, mixed, superactive, thermic Typic Argiustoll			CRP (10 yr)	6.3	149; 241	1.38	9.3	0.81	570
	Madge	Fine-loamy, mixed, superactive, thermic Typic Argiustoll			Crop	7.0	145; 240	1.56	4.0	0.40	200

† (Soil Survey Staff, 1998; USDA-NRCS Soil Survey Division, 2001).
‡ MAT = mean annual temperature.
§ MAP = mean annual precipitation, data taken from local soil survey reports.
¶ CRP = site of the Conservation Reserve Program, that is, formerly long-term cropped soil (≥80 yr) that was taken under fallow for grass reseeding during the last 6 to 10 yr (individual CRP ages given in parenthesis).

Table 2. Land-use effects on bulk density and average organic matter storage in the surface soil (0–5 cm if not differently notified in the heading; $n = 10$ paired sites).

Land use	BD†	C	C	C loss‡ (contents)	C loss§ (stocks)	C loss¶ (adjusted)	C loss§ (5–10 cm)	C/N
	g cm^{-3}	g kg^{-1}	t ha^{-1}	% of native C				
Native	0.93a*	52.3a	215a	100a	100a	100a	100a	10.9a
CRP#	1.30b	25.7b	166a	55b	77b	51b	85a	10.7a
Cropland	1.24b	19.6b	114b	38c	52c	33c	75a	9.7a

* Different letters (a–c) in a column indicate significant difference at the $P < 0.05$ probability level.
† BD = bulk density.
‡ Expressed on a soil weight basis (g kg^{-1}).
§ Expressed on a soil volume basis (t ha^{-1} 0.5 dm^{-1}).
¶ Expressed on a soil volume basis, adjusted for soil compaction at CRP and cropland sites.
# CRP = sites of the Conservation Reserve Program.

6B4, Soil Survey Staff, 1996). Results obtained for total C were not significantly different to those obtained for organic C, as estimated by the modified Walkley Black procedure relying on acid dichromate oxidation (method 6A1c, Soil Survey Staff, 1996), indicating that the surface soil samples were free of significant amounts of carbonates. Hence, total C was considered as organic C. Glucosamine, galactosamine, muramic acid, and mannosamine were analyzed as outlined by Zhang and Amelung (1996). Briefly, samples were hydrolyzed with 6 *M* HCl (8 h, 105°C), filtered, neutralized and converted to aldononitrile acetates. For separation and quantification of the derivatives, a gas chromatograph equipped with a 25-m fused silica capillary column (HP 5, Hewlett Packard, Palo Alto, CA) and a flame ionization detector was used. As Follett et al. (2001) found that CRP effects were most pronounced for the 0- to 5-cm depth interval, we restricted amino sugar analyses to these samples for process identification.

Soil pH was determined in 0.01 *M* $CaCl_2$ in a 1:2 suspension (method 8C1e); particle-size determinations were made on the <2-mm sample by method 3A1 (Soil Survey Staff, 1996) by removing organic matter with H_2O_2, using wet sieving to remove the sand and coarse silt, and the clay and fine silt were separated with the use of a pipette.

Statistics

The impact of land use on soil properties was evaluated using ANOVA followed by post hoc separation of means with the LSD procedure (Statsoft, 1995). Comparison of loss rates between elements were performed as repeated measures in the respective multivariate ANOVA design (Statsoft, 1995).

RESULTS AND DISCUSSION

Breaking native prairie to cropland resulted in significant losses of C and N in the top 5 cm (Table 2), as reported by Follett et al. (2001). The C losses were not related to changes in soil texture or pH ($P > 0.05$). The decline in C storage at the top 5 cm of soil may be attributed to lower organic inputs into the surface horizon, to increased SOM losses by decay and erosion, and to dilution effects by surface mixing, rather than to changes in soil properties.

Dilution effects were evident in reduced C losses when expressing C storage on a volume rather than on a soil weight basis with increased soil compaction at both CRP and long-term cropped sites (Table 2). Sampling the CRP and cropped sites by depth, thus, implied that the top 5 cm of soil comprised material that at the prairie was still located below 5 cm depth. Mathematically adjusting C losses for these differences in bulk density (Ellert and Bettany, 1995) revealed that, on average, two-thirds of the surface soil organic C had been lost during 80 yr of cropping, one-fourth of which being recovered after 8 yr of CRP practice (Table 2), significant only when variability between sites is mathematically reduced by expressing the changes in C storage in percentage of that in the prairie. The C/N ratio remained unchanged in the surface soil, and losses and recovery of soil C reflected those of soil N (data not shown).

Determination of amino sugars promised insight into microbial influences on the organic matter restoration of the upper soil centimeters during CRP practice. The contents of the amino sugars (in mg kg^{-1} soil; Table 1) followed the changes of C and N contents among sites ($r = 0.952$ and 0.951, respectively; $P < 0.001$). To use amino sugars as indicators for a microbial impact on SOM quality, amino sugar contents were expressed in g kg^{-1} C or N.

The amount of amino sugars found at the native sites was similar in magnitude as reported by Amelung et al. (1999) for the top 10-cm soil depth in other sites of the Great Plains. Similar to the finding of Amelung et al. (1999), the amino sugar concentrations (in g kg^{-1} C) increased with increasing mean annual temperature (MAT) in the north-central part of U.S. prairie ($r = 0.63$, $P < 0.05$). Changes in amino sugar concentrations were not related to pH or texture differences between sites ($P > 0.05$).

After 80 yr of cropping, the amino sugar content in the cropland samples was only 24% of that recovered in the native prairie. Relative losses of total N were lower ($P < 0.05$), and N contents in the cropland amounted to 41% of those in the prairie (Fig. 1). The results therefore reflected that amino sugars were lost in preference to other C- and N-containing compounds (see also Zhang et al., 1997), which was confirmed in a decline in the N-normalized amino sugar concentrations from 754 to 452 g kg^{-1} N (Fig. 1). It is concluded that amino sugars in soil do not constitute a stable SOM pool, but may be degraded in preference to other C and N compounds.

Decomposition of microbial products is a general characteristic of substrate shortage for microorganisms in the soil. Lower amino sugar concentrations (in g kg^{-1} C or N) at the cropland sites, therefore, suggested that SOM dynamics at the cropland sites was limited by substrate. A similar conclusion was drawn by Staben et al. (1997) who found that after addition of plant residues

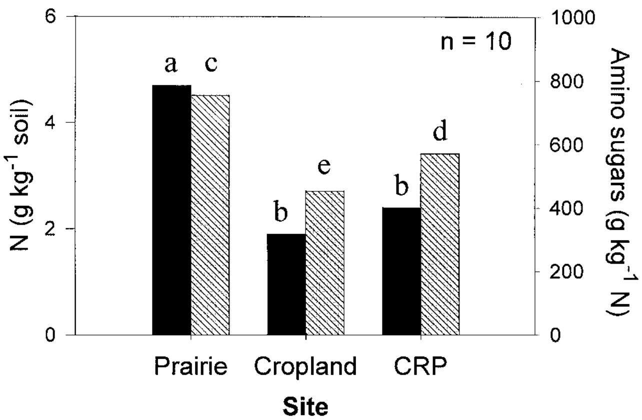

Fig. 1. Average N (left black bars) and amino sugar concentration (right lined bars) in the surface soil (0–5 cm) of the prairie, Conservation Reserve Program (CRP) and cropland fields under study. Different letters (a–b, c–e) indicate significant difference among land-use systems at the $P < 0.05$ level of probability using ANOVA and the respective relative concentrations (normalized to N or the amino sugar content of the prairie) as input parameters.

to two land-use systems, CO_2 production was enhanced in the wheat-fallow relative to the CRP system. Follett and Schimel (1989) reported that increased tillage intensity reduced C availability and, thus, the ability of the soil microbial community to sequester N. Consequently, substrate limitations at cultivated sites do not only favor amino sugar decomposition, but also inhibit amino sugar production by the prevailing microbial community. In this context, amino sugar concentrations can be used as indicators for relative substrate limitations among different land-use systems in a given climate.

Eight years of CRP practice did not result in a significant increase of total topsoil N contents across all sites (Fig. 1), as also found by Robles and Burke (1998). However, CRP significantly increased the relative proportion of N bound in the amino sugar residues relative to the arable soil (Fig. 1). Conservation Reserve Program management thus significantly reduced limitations in substrate, probably due to increased inputs of labile plant-derived C and N pools (Robles and Burke, 1998). On average, CRP restored about one-fourth of the amino sugars lost across 80 yr. The amount of N (and C, data not shown) sequestered in microbial residues can be more easily manipulated by CRP management than the N storage in other sources, such as in particulate plant residues (Robles and Burke, 1998). The time-frame for management effects on the relative proportion of this pool of microbial residues was less than one decade.

Zhang et al. (1997) reported that amino sugars common in bacteria (galactosamine and muramic acid; Parsons, 1981) were lost in preference to glucosamine that is also common in fungi. Also in this study, losses of galactosamine exceeded those of glucosamine ($P <$ 0.05). This resulted in an increased ratio of glucosamine to galactosamine that was more pronounced as the climate warmed (Fig. 2). Whereas Zhang et al. (1997) found that for the top 10 cm of soil depth, total amino sugar losses increased with increasing MAT upon cropping; such effects seemed to be restricted to a relative shift in amino sugar pattern when only the top 5 cm of

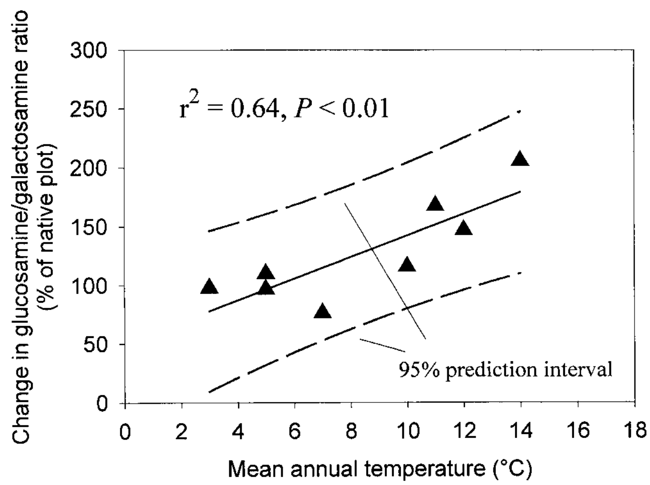

Fig. 2. Relative increase in the ratio of glucosamine to galactosamine in the surface soil (0–5 cm) after long-term cropping of the prairie.

soil were considered as in this study. Nevertheless, it still remains unclear to what extent the increase in glucosamine:galactosamine ratio really indicates a shift of bacterial- to fungal-derived residues in soil (Kögel and Bochter, 1985). The occurrence of galactosamine is not restricted to bacteria since it has been found in some rare fungi and is common in actinomycetes (Sharon, 1965; Herrera, 1992). As reviewed by Amelung (2001), amino sugar ratios may be used as indicators of different microbial origins of residues. However, deducing the contribution of fungal and bacterial residues to SOM from the amino sugar pattern only seemed reliable when changes in glucosamine:galactosamine ratios are consistent with those of the glucosamine:muramic acid ratios. This was not the case in this study. Changes in the ratio of glucosamine:muramic acid did not correlate with MAT ($r^2 <$ 0.1, not significant), but with changes in bulk density (Fig. 3; $r = 0.84$ and 0.78 for the relative changes in this ratio at cultivated and CRP sites, respectively; $P < 0.01$). These results suggest that apart from climate, soil compaction also influenced the capability of different mem-

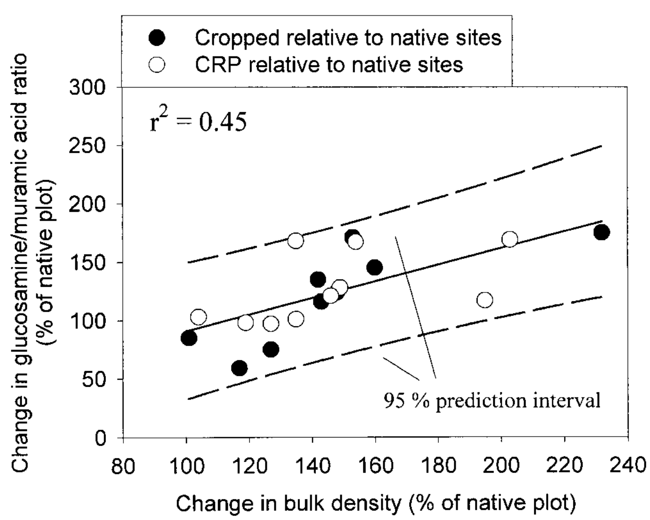

Fig. 3. Changes in the ratio of glucosamine to muramic acid with land use as related to bulk density (0–5 cm).

bers of the soil microbial community to release or to sequester soil C and N under arable and CRP land use, respectively. This is consistent with two previous studies that reported deteriorated conditions for microbially-driven processes in compacted soils (Breland and Hansen, 1996; Ahl et al., 1998).

The native prairie likely represents the climax of C and N sequestration for each of our sites at given climate and inherent soil fertility levels. An efficient restoration of SOM levels under CRP management should require that in addition to the planted botanical composition, the microbial community reflects that of the adjacent prairie. Our data suggest that 8 yr of CRP generally reduced substrate limitations for the soil microorganisms; however, soil compaction still inhibited the sequestration of C and N by muramic acid-containing bacteria. In this context, decompacting arable soil prior to reseeding of grasses might help to improve microbial C and N sequestration, and thus CRP effectiveness.

It should be noted that, in general, soil compaction may result in decreased C and N mineralization rates (Breland and Hansen, 1996; DeNeve and Hofman, 2000). Nevertheless, we would not expect that decompacting degraded arable land would lead to significant N losses, because (i) degraded arable land is already depleted in easily mineralizable nutrient pools (Christensen, 1996), (ii) leaching of nitrate may be assumed to be minimal at semiarid climate and is generally reduced by grass-reseeding at CRP practice (Randall et al., 1997), and (iii) gaseous losses of N are usually lower from better aerated, decompacted soil (Hansen et al., 1993). In contrast, decompaction will improve the conditions for plant growth, either directly or via microbial enhancement of nutrient cycling, which in turn adds more substrate into the system that can be sequestered by the microbial community. For as long as the sites are limited in substrate (Staben et al., 1997), labile plant residues such as particulate organic matter do not accumulate (Robles and Burke, 1998) but are rapidly converted into microbial biomass. In this context, monitoring microbial residues might be superior to monitoring plant residues for assessing CRP efficiency.

CONCLUSIONS

Eight years of CRP practice recovered less than one-fifth of SOM that has been formerly lost upon breaking and continuous cropping (>80 yr) of native grassland soils. However, microbial C and N residues were restored in preference to other organic compounds. The efficiency of CRP management to sequester C and N within a given period of time depends, therefore, on the conditions of how fast and close the pool of living and dead microbial biomass may reapproach equilibrium. Reducing the time span needed to recover this microbial residue pool by fertilization and decompaction prior to CRP might thus warrant further attention.

ACKNOWLEDGMENTS

We thank T. Gonter and A. Rodionov for contributing to the analyses.

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Spatial Modeling of Nitrifier Microhabitats in Soil

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ABSTRACT

Soil bacteria function in the three-dimensional space in heterogeneous soil complex and their activities depend in part on encountering substrates at the microbial scale. The bacterial density per gram of soil, which is generally measured, does not indicate if bacteria are all in the same location or spread throughout the soil complex. We characterized spatial distribution for how dispersed or aggregated nitrifiers (NH_4^+ and NO_2^- oxidizers) were at a submillimeter scale. The spatial approach was based on the relationship, obtained experimentally, between the percentage of microsamples (50–500 μm diam.) harboring nitrifiers and the volume of the microsamples. The smallest sample size (50-μm diam.) was considered as an approximation of microhabitat. The simulated spatial pattern of NO_2^- oxidizer microhabitats in soil were compared with experimental data. The simulated pattern of NO_2^- oxidizer distribution suggested that microhabitats averaged seven NO_2^- oxidizers and occurred in preferentially colonized patches that had about a 250-μm diam. These were randomly distributed and occupied 5.5% of the soil volume. They were functionally connected through microporosity and hence diffusion processes probably controlled the spatial distribution of nirifiers. The nitrifier spatial pattern enabled efficient nitrification because NH_4^+ and NO_2^- oxidizers were near one another. The results showed the potential of our method to study spatial distribution of bacteria at the microhabitat scale.

Bacteria are responsible for major biogeochemical transformations of organic and mineral constituents in soils (Atlas and Bartha, 1981; Paul and Clark, 1989). Soil bacteria live in a complex three-dimensional habitat of a porous heterogeneous medium (Stotzky and Burns, 1982; Tisdall and Oades, 1982; Crawford and Young, 1998; Young and Ritz, 1998). The geometric complexity of soil affects the probability of bacteria encountering appropriate substrates or other bacteria. The quantitative assessment of bacteria in soil is mostly confined to population density or biomass measurements (Atlas and Bartha, 1981; Schmidt, 1982; Powlson, 1994), but rarely is the spatial organization of the cells taken into account (Hattori, 1973). For example, do bacteria in a gram of soil coalesce in a few spots or are they distributed evenly across the soil complex? Dispersion is not available for microorganisms but is routinely measured for macroscopic organisms because it determines the frequency of encountering food and other organisms. Characterizing the spatial distribution of microhabitats is important if there is to be progress in microbial ecology in soils. Also, a better understanding of the spatial arrangement of bacterial habitats should lead to the development of more appropriate bioremediation techniques (increasing probability of bacteria encountering substrates) and the optimization of soil functions (Holden and Firestone, 1997).

Bacterial activities have been reported to be unevenly distributed in soil, leading to the concept of *hot spots* that are linked to local, transient available C for microbial growth and activity (Parkin, 1987; Robertson et al., 1988; Beare et al., 1995). Most microbiological research is carried out on macro scales grams of soil, but bacteria cells exist and interact at the micro scale. Information of the spatial distribution of bacteria in soil is very limited, with microhabitats being poorly defined (Harris, 1994). Hattori (1973) reported results of several early studies on spatial patterns of bacteria in soil and Harris (1994) mentioned that they were mostly based on microscopic observations. The lack of quantitative data on the spatial patterns of bacteria at the microhabitat scale (Hattori, 1973, for total microflora) is because of limitations for sampling and sample processing methods.

The two main locations for active bacteria are believed to be soil pores (Hattori and Hattori, 1976; Hattori, 1988; Pievetz and Steenhuis, 1995), (within the surrounding water film), in regions of preferential flow

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Published in Soil Sci. Soc. Am. J. 65:1709–1716 (2001).

Abbreviations: VU, volumetric unit; MDT, mean detection time.